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EXPERIMENTAL HEMORRAGIC FEVER IN GUINEA PIGS (JUNIN VIRUS)

CONTAGION AND ELIMINATION OF VIRUS*

BY: L. B. DE GUERRERO et al(1)

Although epidemiological, man to man contamination seems not to be significant,[1,2,3] isolated observations have demonstrated that it is possible experimentally.

To confirm this, healthy and inoculated Guinea-pigs were confined in the same cage. Other healthy Guinea-pigs were placed nearby but in different cages.

The airborne contamination and the elimination of virus by feces and urine were also investigated.

The results indicate that contamination by direct contact should be considered at least in the experimental field; that urine is an important elimination via and that airborne contamination is possible.

METHODS AND MATERIALS

Virus.- 16 strains of Junin Virus were used. One, XJ, obtained from inoculated Guinea-pigs (prototype strains[4]) and the others AA/63, BA/63, BT/63, BP/63, ChD/63, DE/63, DiAE/63, FR/63, GR/63, LR/33, MD/63, MJ/63, RR/63, SA/63, ZR/63, obtained by recent isolation[5].

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(1) Investigator for the National Council of Scientific and Technical Research of the Republic of Argentina.

The XJ strain was obtained from plasma (stock 9) and serum (stock 11), both after two passages in Guinea-pigs, 13 in "lauchas" and 22 in Guinea-pigs, with a titer $DL_{50}10^6$.

The other strains, isolated during 1963 epidemic, were passed twice in Guinea-pigs. The inoculating material was a suspension of liver and spleen.

ANIMALS.- Guinea-pigs weighing 250-300 Gms. and newborn white mice were used.

DOSES AND INOCULATION VIAS.- Guinea-pigs were inoculated by injecting I.M. 0.2 c.c.; XJ strain was used in dilution 10^3 and the others in dilution 10^2.

The mice were inoculated subcutaneously injecting 0.02 c.c. of the 10^2 dilution. A M/15, pH 7.4 buffer of phosphate with 2% inactivated serum of normal rabbit, 50 γ of streptomycin and 100 U. of penicillin was used as diluent.

A) For each strain 2 to 10 animals were inoculated and confined in individual cages. Two healthy Guinea-pigs were also confined in each cage. The experience with XJ strain was repeated three times. The normal Guinea-pigs were observed for 50 days after the inoculated ones died. The normal animals died with the characteristic symptoms of the disease in very few opportunities[6]; in general they had injection of the subcutaneous tissue, adrenals, small intestine and inguinal lymph nodes, being the latter frequently hemorragic. This stage was called congestive phase.

The infection was confirmed by passage of liver and spleen of the normal Guinea-pigs that died, to another animal (Guinea-pig and/or newborn mouse) requiring most of the times a blank passage. As Junin virus produces a sufficiently charactersitic set of symptoms in these animals we considered unnecessary a further serologic confirmation.

B) In the same compartment were placed 2 cages, one of them with normal Guinea-pigs and the other wish animals inoculated with XJ strain.

As in A), the normal animals were observed for 50 days after the death of the others.

C) Six Guinea-pigs were inoculated with Junin virus, three of them had plasma (stock 9) and other three had serum (stock 11). All died between 12 and 15 days with the typical symptoms of the disease. Immediately after they died urine and stools were obtained by steril puncture of the bladder and from the large intestine.

The feces in suspension with 10% normal saline were clarified and sterilized by centrifugation. Then Guinea-pigs were inoculated and observed for 50 days.

The urine was diluted in a 10% buffer solution and centrifugated for one hour at 10.000 rpm in a Serval SSI centrifugal machine at 0° C. The pH of the fluids after centrifugation was between 6.5-7.5. Since they were within the limits of viral viability, they were not neutralized.

0.5 ml of each material in 10^1 dilution were inoculated after being sure of checking on its bacteriological sterility.

In view of the results (Table 3) it was decided to investigate when the Guinea-pigs start to eliminate virus through the urine.

Thus, from a group of 30 animals inoculated with XJ (stock 11), 2 were killed daily. The urine of this Guinea-pigs were injected into other 2 following the technique described above. This animals were observed for a month.

D) Seven Guinea-pigs were placed in a nebulization apparatus and nebulized[7] with 8 c.c. of a dilution 10^3 of the XJ strain for 15 minutes. Before taking them out the apparatus was sterilized with L.U.V. for 10 minutes.

RESULTS

Fifty-six animals were inoculated with 16 strains of Junin virus (Table 1).

TABLE 1

Normal Guinea-pigs infected by direct contact with others inoculated with different strains of Junin virus

Strains	Number of Guinea-pigs inoculated	Normal Guinea-pigs	
		Number of animals	Number of positives
XJ stock 9	3	4	1
XJ stock 11	4	4	-
XJ stock 11	2	2	-
XJ stock 11	2	2	-
XJ stock 11	2	2	-
AA	6	2	-
BA	2	2	1
BT	2	2	-
BP	4	2	-
ChD	2	2	-
DE	5	2	-
DiAE	2	2	1
FR	2	2	-

Strains	Number of Guinea-pigs inoculated	Normal Guinea-pigs	
		Number of animals	Number of positives
GR	4	2	1
LR	2	2	-
MD	2	2	-
MJ	2	2	-
RR	1	2	1
SA	1	2	-
ZR	6	2	-
Totals	56	44	5

XJ is a strain adapted to Guinea-pigs, whereas the other strains have been recently isolated. Five out of 44 normal animals exposed to the infection died. They were those exposed to XJ (stock 9), BA/63, DIAE/63, GR/63 and RR/63. It was possible to determine the viruses by successive passages.

These 5 deaths represent 11.3% of all Guinea-pigs exposed to infection. Each one corresponds to a different strain, which means, that 31% of all the strains were able to produce infection.

This, also, demonstrates that there is 11.4% of probability for a Guinea-pig to be infected by direct contact with inoculated animals. Because of the number used by us these figures may vary from 1.8% to 21%.

The normal Guinea-pigs in the same compartment, but in different cages, were all healthy (Table 2).

TABLE 2

Contagion between animals inoculated with Junin virus, XJ strains, and normal Guinea-pigs placed in separated cages

Box	Inoculated animals	Normal animals	
	Number of animals	Number of animals	Number of positives
1	2	4	-
2	2	4	-
3	2	3	-

TABLE 3

Isolation of virus from urine and feces of Guinea-pigs inoculated with Junin virus, XJ strain

Material	Number of samples	Number of positives
Urine	6	6
Feces	6	-

It was not possible to isolate any virus from feces (Table 3). There were viruses in all the urine samples taken once the animals died (Table 3). Consecutive samples taken every day from live animals demonstrated virus after the 7th day of inoculation (Table 4).

TABLE 4

Daily isolation of Junin virus (XJ strain) from urine of inoculated Guinea-pigs

Days after inoculation	Number of inoculated animals	Isolation of virus from urine*
1	2	0/2
2	2	0/2
3	2	0/2
4	2	0/2
5	2	0/2
6	2	0/2
7	2	2/2
8	2	2/2
9	2	2/2
10	2	2/2
11	2	2/2
12	2	2/2

*Number of deaths over number of inoculated animals.

All the animals exposed to nebulization became infected and died with the typical symptoms of AHF.

DISCUSSION

The results suggest that there is a possibility of producing experimental infection between inoculated and normal animals by direct contact. Both the adapted and the recently isolated strains behave in the same way. However, it was observed that the animals infected by XJ strain that died always presented the symptoms of AHF, whereas in the other animals, subsequent passages were necessary in order to demonstrate the virus.

It is worth remembering that we consider positive contagion only when the animal dies and the death is due to the virus itself. This is confirmed either by the characteristic signs or by successive passages. By doing so, we do not discard the possibility of hidden infections that we decided not to take into consideration.

That close contact is necessary for contamination is demonstrated by the fact that the animals placed in neighboring cages were not infected.

The repeated isolation of viruses from urine of Guinea-pigs dying of hemmorragie fever as well as from two human cases, indicate that this is an important via of virus elimination and may be a vehicle for contamination. However, it was not possible to isolate virus from stools.

The virus was isolated from the urine of infected Guinea-pigs since the 7th day after inoculation to the day of their death. In other words, the elimination starts several days after the viremia is established. On the other hand, air-borne infection has proven to be highly effective in experimental fields.

Therefore, we can state that contamination by close contact is very important, at least in the experimental field.

There is no doubt that the virus is eliminated by urine and that the air-borne via infects the Guinea-pigs easily. This does not exclude other ways of contagion. We do believe that all this should be kept in mind in the care and handling of patients and experimental animals.

CONCLUSIONS

1) Contact contamination is possible and should be considered whenever the animals are closely confined in the laboratory. We have not found any difference between strains of viruses whether they were or not adapted to Guinea-pigs.

2) The virus is isolated from urine of infected animals from the 7th day after the inoculation.

3) The virus is not eliminated through fecal material.

4) Air-borne infection occurs in Guinea-pigs.

SUMMARY

In this paper are studied the possibility of contamination between infected and normal guinea pigs, the infection of guinea pigs by air borne-route and the elimination of virus by urine and stools of infected animals.

Normal guinea pigs were exposed to infection by placing them in the same cage and in neighboring ones to the cages of inoculated guinea pigs (16 strains of Junin virus were employed).

The 100% of inoculated guinea pigs died with the typical signs of AHF. Only the 11.3% of normal guinea pigs placed in the same cage were infected. This means that the 31% of the strains employed contaminated the normal animals. None of the normal animals placed in neighboring cages died.

By inoculating guinea pigs with daily urine samples taken from guinea pigs infected with Junin virus (strain XJ) was find that the virus appears in urine from the 7th day after the inoculation.

The virus was not isolated from stools of infected animals.

Four guinea pigs were infected by nebulization with a suspension of the virus. The animals died with the typical signs of AHF.

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